AMD-like Lesions in the Rat Retina: A Latent Consequence of Perinatal Hemorrhage

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Purpose. Hemorrhaging is a commonplace event in the human retina around the time of birth. This study was conducted to examine the potential long-term sequelae of hemorrhaging in the eyes of rats that exhibited transient spontaneous microhemorrhages a few days after birth.

Methods. Retinas of Dark Agouti rats aged from day of birth to 2 years old were analyzed histologically, histochemically, and by immunocytochemistry. Fetal human retinas were also examined anatomically and histochemically for evidence of hemorrhages.

Results. Dark Agouti rats from our colony consistently exhibited spontaneous focal hemorrhages at the vitread surface of the retina between postnatal days 3 and 6. Erythrocytes were subsequently cleared by macrophages, which accumulated hemosiderin. These macrophages remained in focal patches in the inner retina for the duration of the study. For at least 6 months after the initial transient hemorrhages, the retinas exhibited no overt histologic damage. At ~8 to 9 months, photoreceptor degenerative changes were apparent in spatial register with the patches of macrophages in the inner retina. Additional events such as breakdown of Bruch's membrane, glial remodeling, neovascularization, ingress of RPE cells into the retina and accumulation of drusen-like autofluorescent structures were also observed in topographic register with macrophage-laden patches in aged animals.

Conclusions. Microhemorrhages in the retina may initiate the formation of focal lesions, months or years after the initial insult. The lesions exhibit key features of AMD. These animals may represent a useful model for studying the potential basis of the pathogenesis of AMD. (*Invest Ophthalmol Vis Sci.* 2008;49:2790–2798) DOI:10.1167/iovs.08-1757

The purpose of the present study was to examine the potential consequences of hemorrhage in the developing retina—in particular, whether this was associated with immediate or delayed degenerative changes. The rationale for the study was the prior observation of a very high incidence of retinal microhemorrhages in term and preterm infants. Microhemorrhages have been detected in approximately one third of newborn infants.[1,2] Trauma to blood vessels that are innately fragile during early development may account for the >75% incidence in infants delivered with vacuum assistance.[2] Other sources of trauma, such as child abuse, may also contribute to retinal hemorrhage in the newborn infant.[3] Other factors not related to trauma that may also cause retinal hemorrhages include retinopathy of prematurity, vitamin K and C deficiencies, clotting factor deficiencies, deficiencies in anticoagulants such as protein C, and maternal smoking. More broadly, other agents that may give rise to retinal hemorrhages include infections such as dengue fever, cytomegalovirus, measles and malaria, axial myopia, and raised or rapidly lowered intraocular pressure.[4–10] Despite the extremely high incidence of perinatal retinal hemorrhages, there is remarkably little data concerning the possible consequences of such hemorrhaging in the premature or term neonate. A common view is that these hemorrhages resolve within 2 weeks, and have little effect on the eye as assessed using measures such as visual acuity and contrast sensitivity over periods of months[11] to a few years.[12,13] This led to the generally held view that neonatal retinal hemorrhages are common and benign.[14]

However, there is a minority converse opinion. It has been suggested that hemorrhages involving the macula may evoke degenerative changes or an exudative or glial scar as well as macular pigmentary disturbances, albeit in a very small number of individuals.[15] It remains to be determined whether the relatively normal vision commonly reported in individuals who have experienced macular hemorrhage is attributable to lack of damage, or perhaps to the extraordinary plasticity that is evident in the immature human visual system.[16]

A rather different outcome is evident in the case of hemorrhaging into the subretinal space. It is known that in a rodent model of retinopathy of prematurity, where newly formed vessels have a tendency to hemorrhage, blood accumulates in the subretinal space and is associated with the formation of photoreceptor rosettes and retinal detachment.[17] This retinopathy is of interest, not only because of its frequent clinical presentation in premature infants,[18] but also in the context of the present study, as there seems to be a strain dependence in susceptibility. Thus, pigmented rat strains are generally more susceptible than albino strains.[19,20] The damage to photoreceptors is probably a consequence of the hemorrhaging, since autologous serum, when injected into the subretinal space, also causes photoreceptor death. Because the intercalation of photoreceptor outer segments and the processes of RPE cells is the key anatomic substrate for retinal attachment to the RPE, photoreceptor death may influence detachment of the retina from the RPE.[21]

To date, an effective experimental model has not existed for examining the consequences of hemorrhage within the neonatal retina in the absence of other overt initial disease. In this study, the fortuitous finding of a novel animal model is re-

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ported. It is demonstrated that Dark Agouti rats from our colony are unusual in that, unlike other rat strains that are commonly available, these rats consistently exhibit small spontaneous retinal hemorrhages during early postnatal development. Parallel observations were also made on human fetal retinas to determine whether comparable hemorrhages might be evident in retinas that were at an equivalent developmental time point.

Materials and Methods

This study adhered to the tenets of the Declaration of Helsinki for research involving human subjects and was in strict accord with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The number of animals used was minimized, as was any potential distress or discomfort to the animals. All experiments were performed in accordance with NHMRC (National Health and Medical Research Council, Australia) guidelines and approved by the appropriate institutional ethics committees.

Animal Experiments

Dark Agouti rats were from an inbred isolated colony. The animals were housed under standard conditions and given access to food and water ad libitum.

Animals (n = 94, both male and female) aged from postnatal day 1 to 2 years were killed by overdose of pentobarbital sodium (100 mg/kg

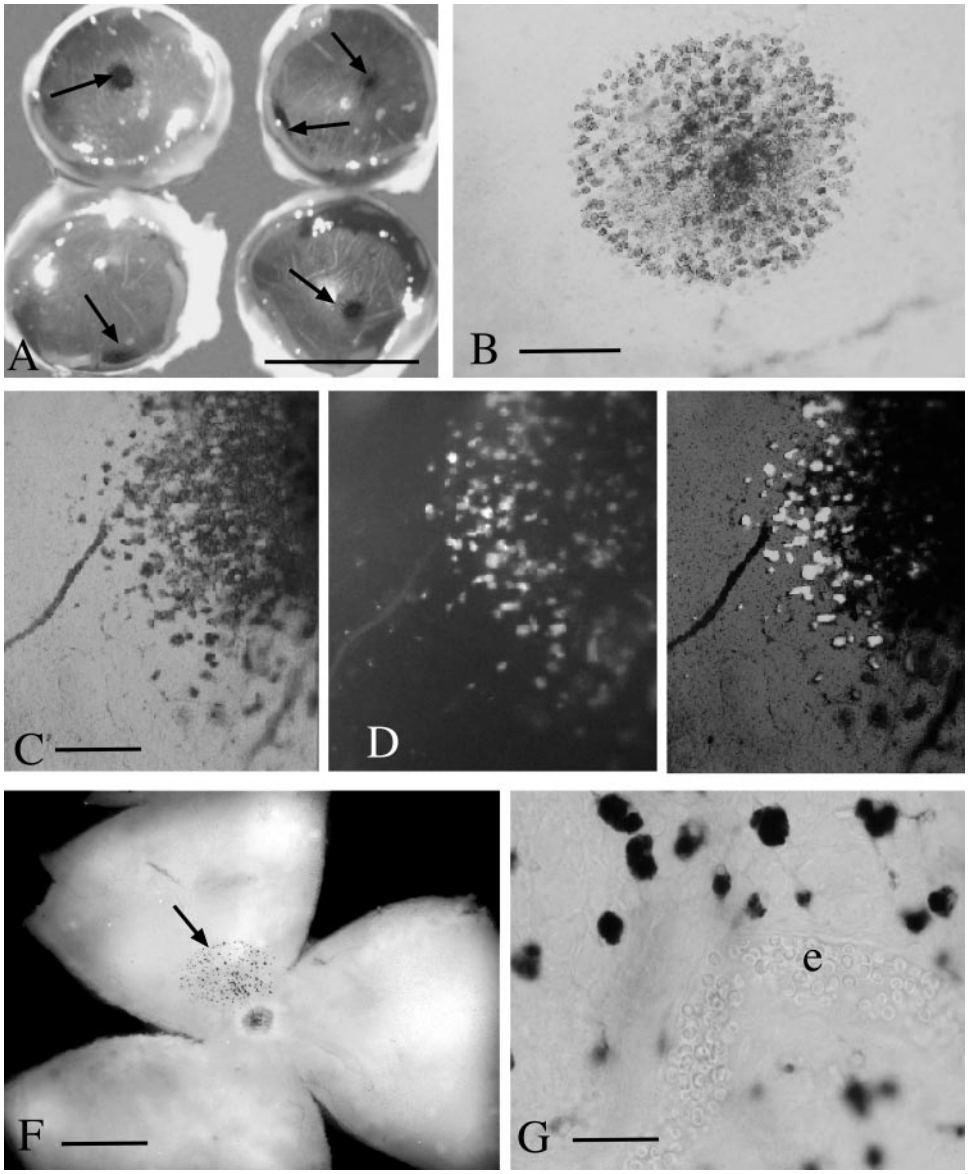

Figure 1. (**A**) Eyecups from four animals aged 5 days postnatum. Each eyecup typically exhibited one hemorrhage (*arrow*), but occasionally two or more were present. (**B**–**G**) Wholemounted retinas from animals aged postnatal days 6 (**B**) and 8 (**C**–**E**). (**F**, **G**) Retinas from animals at postnatal day 21. Patches of erythrocytes were still evident at day 6. By day 8, macrophages appeared at the periphery of the hemorrhages. Superimposition of bright-field images (**A**) with a fluorescence image showing labeling for OX42 (**B**) revealed the localization of macrophages at the periphery of the hemorrhages (**C**). Staining using Perl's method (**F**, **G**) confirmed that the patches of macrophages contained hemosiderin, as evinced by the presence of intense blue staining in the cells (**F**). Conversely, erythrocytes (e) were not stained by this method (**G**). Scale bars: (**A**) 2 mm; (**B**) 0.25 mm; (**C**) 50 μm; (**F**) 500 μm; (**G**) 25 μm.

Table 1. Incidence of Hemorrhages and Macrophage Patches

Age of Animals	Eyes Examined (*n*)	Number of Hemorrhages or Macrophage Patches			
		0	1	2	3 or More
1 d	12	12	0	0	0
3 d	18	2	14	2	0
5–6 d	18	1	13	3	1
7–10 d	18	2	12	4	0
21 d	18	1	15	2	0
3 mo	18	0	15	2	1
6 mo	18	2	13	3	0
8 mo	16	1	12	3	0
10 mo	16	2	11	3	0
12 mo	16	3	10	2	1
18 mo	10	1	11	4	0
2 y	6	1	5	0	0

IP). Eyes were enucleated, the cornea and lens removed, and the eyecups fixed by immersion with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 to 2 hours. Eyes were then examined macroscopically for evidence of hemorrhages or lesions. Retinas were then removed from the eyecups, either in isolation, or with the overlying RPE and choroid attached. Fluorescein angiography was also performed on a small subset of eyes (1 each of animals aged 6 days, 21 days, and 6 months of age) using standard methods[22] to determine the patency of vessels at the sites of hemorrhages.

Retinas were initially examined as wholemounts, by bright-field and fluorescence microscopy, to enable identification of the presence of microhemorrhages, patches of hemosiderin-containing macrophages and any other histologic anomalies that might be evident. The incidence of hemorrhages and macrophage patches is summarized in Table 1. A subset of retinas (two each from ages 4, 10, and 21 days and 6 months) were stained histochemically by Perl's method, to detect hemosiderin, the fixed retinas being stained in a mixture containing 1% potassium ferrocyanide and 1% hydrochloric acid for 5 minutes, thereby forming the Prussian blue reaction product in cells containing hemosiderin. Other retinas that were not stained for hemosiderin, were then counterstained with the fluorescent nuclear dye DAPI, according to standard methods,[23] to allow counts of cell nuclei in the ganglion cell layer. Thereafter, tissues were dehydrated and embedded, ether in paraffin wax (paraplast) or Araldite epoxy resin according to standard methods[24,25] and sections stained with cresyl violet or toluidine blue.

Immunocytochemistry was subsequently performed on wax sections with antibodies against the glial cell intermediate filament protein GFAP (Sigma, Castle Hill, Australia) or the glial glutamate transporters GLAST (raised in house), by standard methods,[26] to determine any changes in glial cell anatomy or function. Macrophages were identified with the MRC OX42 monoclonal antibody (Serotec, Sydney, Australia) against CD11 on wholemounts of retina, by standard methods[27] with a biotinylated anti-mouse secondary antibody and the labeling revealed by streptavidin conjugated to Texas red (GE Healthcare, Castle Hill, Australia).

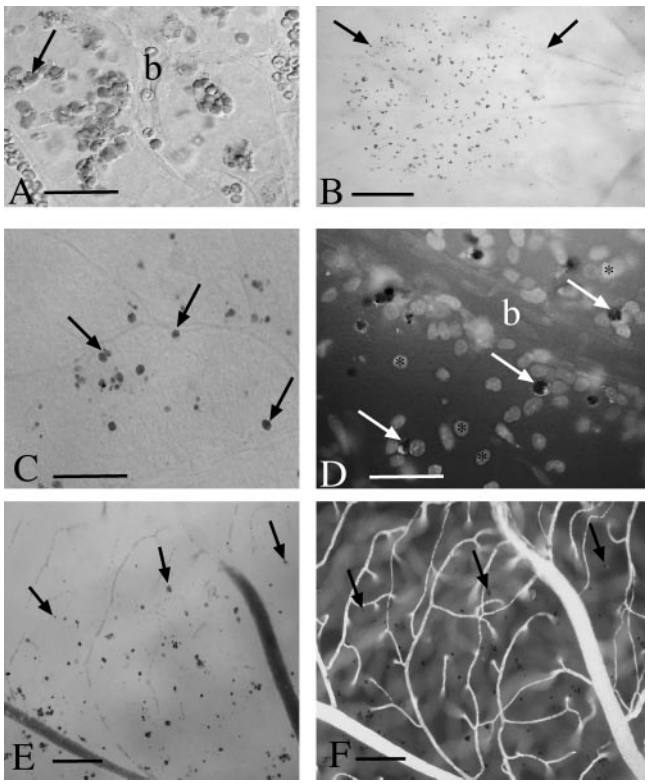

Figure 2. Images from animals at postnatal day 10 (**A**) and 6 months (**B–F**). Macrophages appeared to remove erythrocyte debris, resulting in their content of yellow-brown hemosiderin. Patches containing macrophages were still prominent at 6 months of age (**B**, **C**). DAPI staining (**D**) confirms nuclei of ganglion cells and other cell types remained in the macrophage-containing patches. (**E**, **F**) Paired bright-field and fluorescence images of a portion of retina at the edge of a patch of macrophages (*arrows*), demonstrating that vessels within the hemorrhage regions remained patent, as assessed by fluorescein angiography. B, blood vessel. Scale bars: (**A**) 25 μm; (**B**) 0.5 mm; (**C**, **E**, **F**) 50 μm; (**D**) 30 μm.

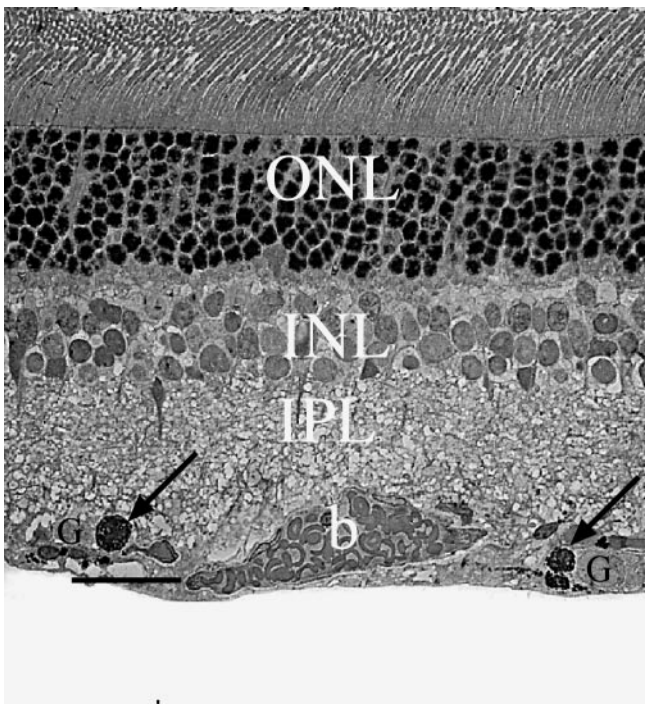

Figure 3. Transverse resin-embedded section of rat retina aged 6 months stained with toluidine blue. Hemosiderin-containing macrophages (*arrows*) are present, but there appeared to be no anomalies in the outer nuclear layer (ONL), inner nuclear layer (INL), or inner plexiform layer (IPL). Blood vessels (b) were patent. G, Ganglion cell somata. Scale bar, 25 μm.

Human Tissues

Human fetal retinas ($n = 3$) were collected with maternal consent from therapeutic pregnancy terminations and gestational ages estimated at 18.5 weeks, from ultrasound and measurements. Fetal tissues were collected within 20 minutes to 2 hours after termination and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) by immersion for 3 hours. Tissues were examined initially using bright-field microscopy, and then again after staining for hemosiderin by Perl's method.

Results

All data were comparable for both male and female rats; accordingly sex is not specified in the results.

Analysis of retinas from animals aged 3 days revealed the presence in all animals, of small punctate hemorrhages, typically ~0.5 mm in diameter at the vitreous surface of the retina, that formed at the leading edge of the newly forming vasculature of the retina. There was no evidence of bleeding into the vitreous humor, indicating that the inner limiting membrane of the retina was probably intact. Hemorrhages were still prominent in all retinas examined at postnatal day 5 (Fig. 1A); retinas typically exhibited a single hemorrhage, but sometimes two or more hemorrhages were evident. Hemorrhages were still prominent at 6 days postnatum (Fig. 1B), but the erythrocytes were clumped, suggesting that the hemorrhaging had ceased at an earlier time point.

By ~7 to 10 days (Figs. 1C–1E) OX42 immunoreactive macrophages appeared at the periphery of the hemorrhages and developed pale yellow-brown hemosiderin-like inclusions that could be stained an intense blue by Perl's method (Fig 1F, 1G), indicating the presence of hemosiderin. By contrast, erythrocytes that contain hemoglobin were not stained (Fig. 1G).

In the absence of histochemical staining, these macrophages could still be seen in bright-field examination of isolated retinas with transmitted light, particularly where they were coexistent with erythrocytes (Fig. 2A). By 14 to 21 days postnatum, erythrocytes disappeared in the hemorrhage region (aside from those within blood vessels), due to the phagocytosis of the cells by the macrophages and the consequent conversion of the red-brown hemoglobin to the much paler hemosiderin. This process created difficulty in observing the patches of macrophages longitudinally, because the pale hemosiderin was not visible by reflected light microscopic techniques, either in the eyecups when observed via a dissecting microscope or in vivo, via an ophthalmoscope. However, the macrophages remained readily visible when viewed with transmitted light because of the light absorbance conferred by hemosiderin and by histochemical Perl's staining.

Prominent patches of hemosiderin-containing macrophages comparable to those first seen at the sites of retinal hemorrhages were still evident in all animals at 6 months (Fig. 2B). These macrophage patches were assumed to represent the sites of the initial hemorrhages because we saw no evidence of progressive loss of macrophages at interim time points, or for any new hemorrhages. Similarly, prominent patches of macrophages were evident at all later time-points in all animals.

The incidence of microhemorrhages and patches of macrophages at each age point examined is summarized in (Table 1).

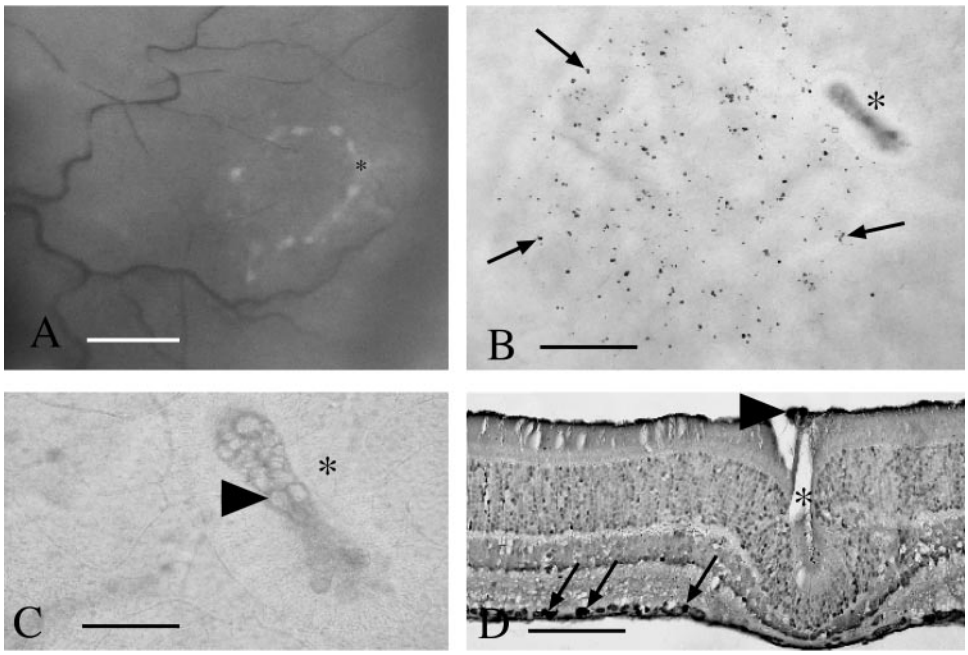

Figure 4. Eight-month-old rats. (**A**) Macroscopic image showing hyperreflective patches (✻) forming an annulus in the retina. (**B**) Wholemount of a similar retina showing the presence of a patch of hemosiderin-containing macrophages (*arrows*) and at the margins of the patch, an anomalous structure in the deeper layers of the retina, representing the hyperreflective structures indicated in (**A**). (**C**) Higher magnification of the anomalous structure in (**B**) focused at the layer of the photoreceptors. The photoreceptors appeared to be distorted, and their outer segments were directed horizontally. Macrophage-like cells were located at the center of the structure. (**D**) Transverse wax section through a structure of the type illustrated in (**A–C**), stained with cresyl violet, depicting a typical rosette structure (✻). Macrophages were present (*arrows*) at the vitread surface and also at the center of the rosette on the outer retinal surface (*arrowhead*). Scale bars: (**A**) 0.5 mm; (**B**) 200 μm; (**C**, **D**) 100 μm.

These data suggest that the number of microhemorrhages observed in each retina at early ages (3–21 days) would be consistent with the numbers of macrophage patches observed at later ages. High-magnification analysis of wholemounts (Fig. 2C) suggested that the hemosiderin-containing macrophages were located almost exclusively in the superficial nerve fiber layer or ganglion cell layer.

Analysis of DAPI-counterstained sections with fluorescence microscopy (Fig. 2D) showed the presence of numerous dark macrophages (the hemosiderin pigment absorbs the UV light used to excite the DAPI) surrounded by nuclei of many other cell types in the ganglion cell layer. DAPI-labeled cell types included ganglion cells, and other cell types such as endothelial cells (with flattened elongated nuclei) and displaced amacrine cells (with small, round nuclei). No significant difference was detected in the number of DAPI-positive cells in the ganglion cell layer when counts were performed in the areas containing macrophages and in areas immediately adjacent, at the same eccentricity (data not shown), suggesting that the ganglion cells and other cell types in the ganglion cell layer were not overtly killed as a consequence of the retinal hemorrhages.

To verify that the blood vessels were patent in the hemorrhage regions, fluorescein angiography was performed. Angiography demonstrated (Figs. 2E, 2F) that vessels in the region of the hemorrhages remained patent, thus indicating that the hemorrhage area remained vascularized, and the tissues were supplied with blood.

Examination of retinas from animals aged around 6 months, revealed no apparent subsequent anomalies in the anatomy and histology of the retina except for the presence of the hemosiderin-containing macrophages (Fig. 3). At this age there was no overt evidence of photoreceptor degeneration. Between 7 and 9 months postnatum, prominent changes started to become apparent in the retinas. Annuli formed by hyperreflective spots were observed during macroscopic examinations of the eyecups (Fig. 4A), and close examination of the retinal wholemounts revealed that at the precise margins of the macrophage-containing patches, anomalous structures (representing such reflective patches) formed in the outer retina ((Figs. 4B, 4C). In transverse section these structures were identified as rosettes, formed by involutions of the outer nuclear layer and characterized by loss of photoreceptor outer segments (Fig. 4D). Macrophage-like cells frequently clustered at the outer surface of the rosette (Figs 4C, 4D). Conversely, degenerative features were not observed at loci that were not in spatial register with patches of macrophages.

Immunocytochemistry for the Müller cell marker GLAST (Fig. 5A) revealed anomalies in the Müller cells, with focal upregulation of GLAST in areas where degenerative anomalies were evident. Similarly, GFAP expression was upregulated at such sites (Fig. 5B).

A key feature evident at sites of degeneration was the breakdown of the blood–retina barrier (BRB) as evinced by breaks in Bruch's membrane and the loss of RPE cells, or the loss of their typical flattened monolayer appearance (Fig. 5C). This loss of anatomic features indicative of barrier function was most noticeable in the oldest animals examined (18 months–2 years), where anomalies in the RPE were consistently evident, with dark patches of RPE apparently penetrating the retina (Fig. 6), typically at the edges of macrophage patches in the inner retina (Fig. 6B). Close analysis of such sites suggested that in addition to breakdown of the BRB, large blood vessels from the choroid appeared to penetrate the retina, in a process suggestive of choroidal neovascularization and chorioretinal anastomosis. Inner retinal vessels within the

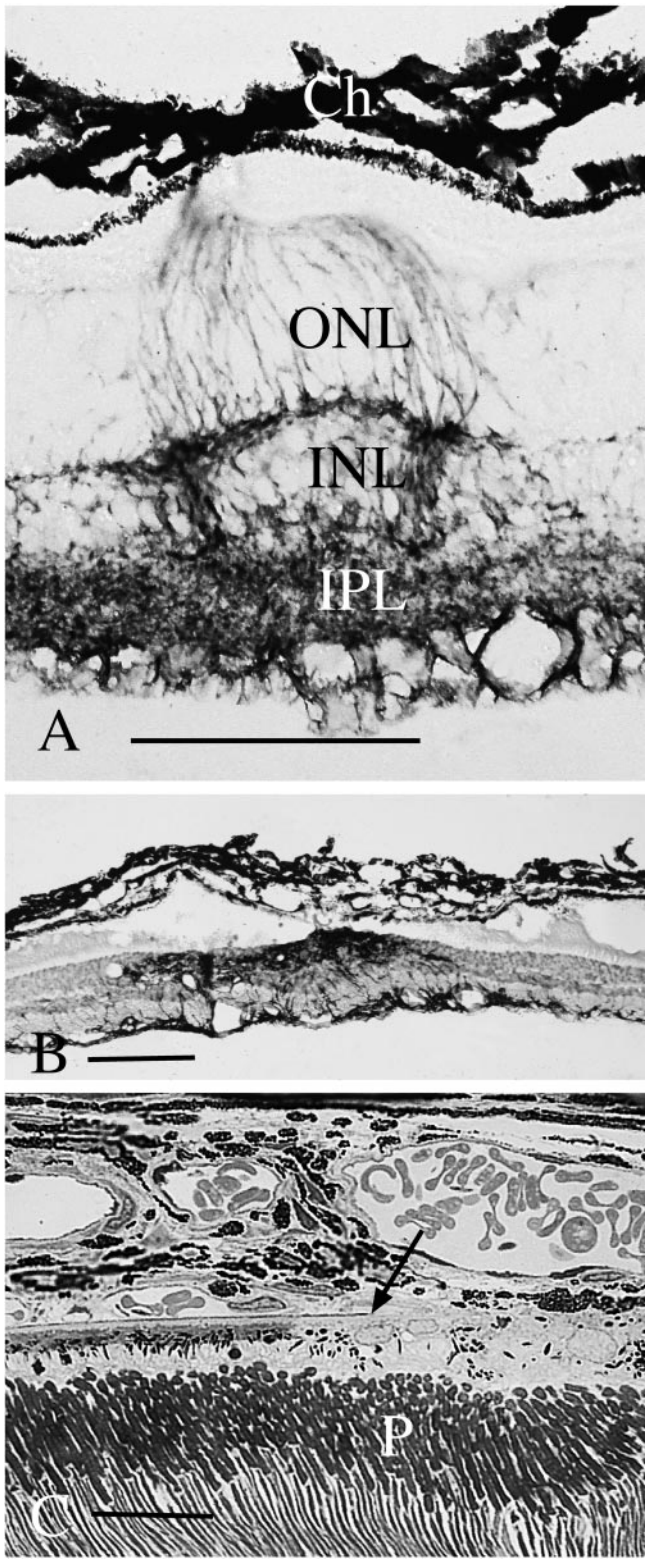

FIGURE 5. Rats aged 10 months. GLAST (**A**) and GFAP (**B**) labeling was upregulated in those retinal regions exhibiting formation of rosettes or other foci of photoreceptor degeneration. (**C**) Breakdown of Bruch's membrane (*arrow*) in a region of retina without apparent photoreceptor disease. Ch, choroid; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; P, photoreceptor outer segments. Scale bars: (**A**, **B**) 100 μm; (**C**) 10 μm.

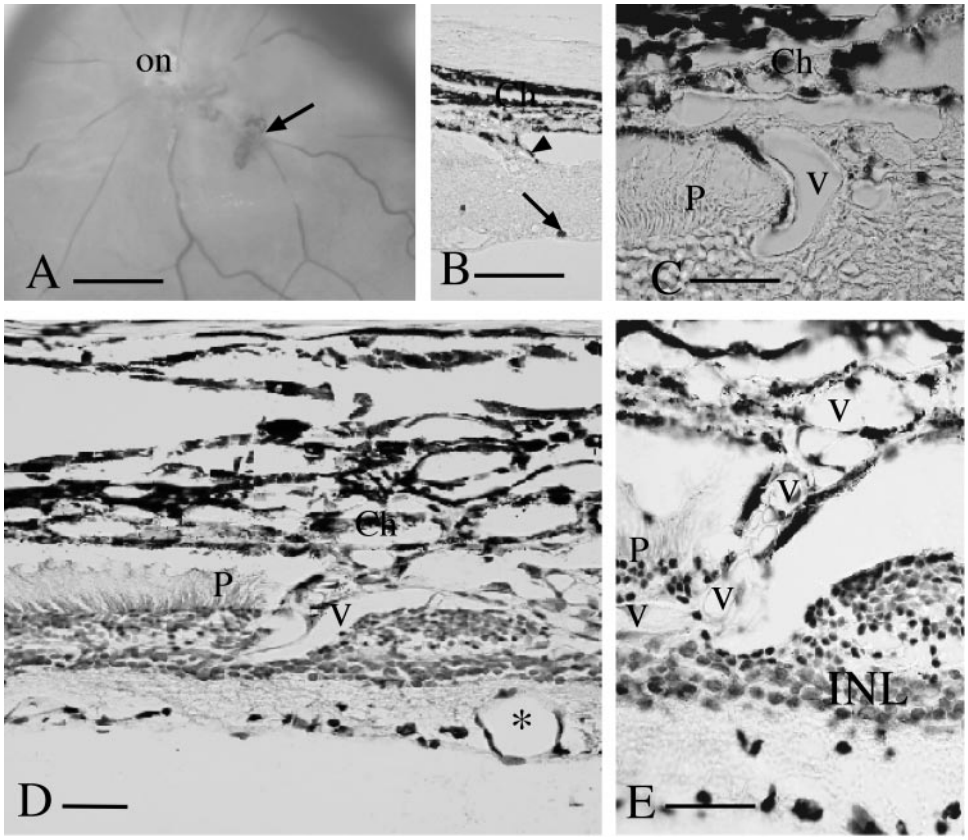

FIGURE 6. Rats aged 18 months. (**A**) Eyecup showing dark pigmentary disturbance that appears to penetrate the retina (*arrow*). (**B**) Transverse section showing a similar pigmentary disruption. An invaginating area of RPE was evident at the margins of an area containing macrophages (*arrow*). (**C**) Higher magnification showing the invaginating pigmented region was associated with a small blood vessel (v). To the *left* of the vessel, photoreceptors (p) were intact; to the *right*, they were missing. (**D**, **E**) Cresyl violet–stained sections showing photoreceptors at the edge of the lesion site and choroidal vessels that appeared to penetrate the retina within the lesion area. Inner retinal vessels (*) appeared to be distended. The inner nuclear layer (INL) appeared to be intact. Scale bars: (**A**) 0.5 mm; (**B**) 100 μm; (**C**) 20 μm; (**D**) 50 μm; (**E**) 25 μm.

damaged region also appeared distended (Fig. 6D). Within areas of BRB breakdown, photoreceptor loss was conspicuous (Figs. 6C–6E).

An additional hallmark of AMD is the accumulation of autofluorescent drusen; the retinas were examined for evidence of drusen. In animals aged 8 months and older, it was observed that at sites where macrophage patches were evident, outer retinal elements exhibiting bright autofluorescence could be detected (Figs. 7A–7C). Careful analysis of the tissues suggested that some of this drusen-like fluorescence was potentially attributable to macrophage-like cells containing fluorescent inclusions. Many of these cells were closely apposed to the outer segments of the photoreceptors (Figs. 7D, 7E). Nevertheless other lipid body–like inclusions observed in the overlying choroid (Fig 7F) may also have contributed to these drusen-like fluorescence signals.

To determine whether the hemorrhages that were observed in the neonatal rat might be of relevance to human retinas, three human fetal retinas were examined. In two of the three retinas, small hemorrhages were identified on the vitread surface of the retina that were comparable to those observed in rat retinas (Fig. 8A). It was recognized that these small hemorrhages might represent perimortem artifacts associated with the termination procedure; therefore, these retinas were stained for hemosiderin. Hemosiderin deposits cannot form postmortem as they require the active phagocytosis of erythrocytes and the metabolic conversion of hemoglobin to hemosiderin. Hemosiderin-containing macrophages were observed in the fetal human retinas (Fig. 8B).

DISCUSSION

In this study, it was demonstrated that neonatal hemorrhages that were associated with the inner retina of the rat appeared to give rise to changes in the outer retina, in close spatial register, albeit with an extreme latency of many months or possibly years. Accordingly this study had two key outcomes. First, it demonstrated that neonatal hemorrhage may not be a benign event but may instead have long-term sequelae. Second, it demonstrated a biologically relevant model for the study of formation of AMD-like lesions, since microhemorrhaging is a common event in the nervous system, and is a key mediator in the formation of features such as plaques in Alzheimer disease.[28]

The proposal that hemorrhaging may be a component in the pathogenesis of AMD accords well with findings that allelic variations in complement factor H genes are the most significant risk factors yet identified for AMD.[29–32] In the CNS, complement factor H is produced by glial and retinal pigment epithelium cells[33,34] and is critical in modulating inflammatory

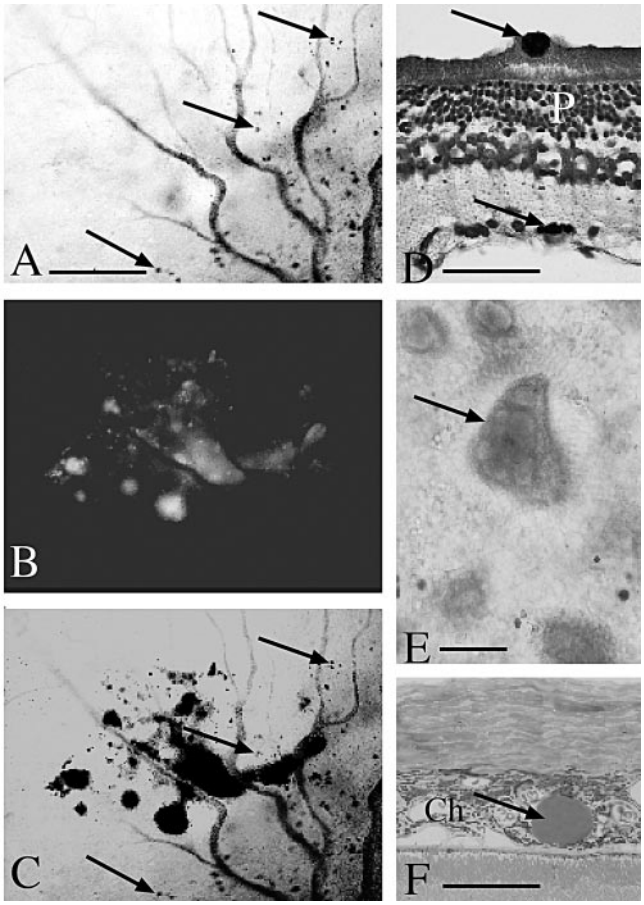

FIGURE 7. Rat retina aged 1 year. (**A**) Bright-field image showing blood vessels. The approximate boundary of the macrophage patch is indicated by *arrows.* (**B**) Fluorescence image of the same field, showing autofluorescent structures. (**C**) Composite of (**A**) and (**B**) superimposing the two grayscale images (the image map of **B** is inverted, to aid in photographic reproduction); the drusenlike accumulations are present at the periphery of the macrophage patch. *Arrows* indicate the positions of macrophages indicated in (**A**). (**D**) Transverse section and (**E**) view from the outer retinal surface showing the presence of macrophage-like structures embedded in the outer segment region of the photoreceptors. These macrophages and associated debris appear to represent the fluorescent structures depicted in (**B**). (**F**) Lipid-like bodies (*arrow*) in the choroid (Ch) are also present, but it is uncertain if they contribute to the autofluorescent drusenlike signal. Scale bars: (**A–C**) 200 μm; (**D**, **F**) 50 μm; (**E**) 25 μm.

responses after hemorrhage.[35] Similarly, smoking and elevated plasma homocysteine levels, both of which each cause vascular perturbation, are also major risk factors in AMD.[36]

The origins of the hemorrhages that are observed in this study are unclear, since they are not evident in other rat strains that were investigated in any of our earlier studies of retinal development, and have not been reported in the literature. They are clearly not the consequence of birth trauma's acting on the fragile vessels of the neonate, since the rat retina is essentially avascular at birth, with the vessels growing out over the surface of the retina in the few days after birth.[37] In this respect, retinas from rats aged around postnatal days 3 to 5 are developmentally equivalent to retinas from human fetuses of ~18 to 20 weeks' gestation. Our demonstration that similar small hemorrhages are evident in the central retina of a small number of human fetal retinas at a comparable developmental time point, when the peripheral retina is not yet vascularized,[38] suggest that our results may be directly pertinent to human retinal development and possibly dysfunction, but this requires confirmation with a larger sample size.

The rapid appearance of macrophages in response to CNS hemorrhaging is not unexpected, in that macrophage infiltration is typically noted within 1 to 3 days.[39] The persistence of macrophages at sites of hemorrhaging in the human and rodent brain is a routine finding,[40] and this as been confirmed in the retina, where hemosiderin-containing macrophages persist for many months to years.[41] Although they are a convenient anatomic marker of the sites of hemorrhaging, it is probable that macrophages also influence the development of retinal disease. Mice deficient in monocyte chemoattractant protein 1 (MCP1; also known as Ccl2) or its receptor (ccr2) develop features of AMD.[42] Similarly, in a recently described mouse model, deficiencies both in Ccl2 and the chemokine receptor cx3cr1 on microglial cells give rise to AMD-like features.[43] This finding accords well with recent human data showing that homozygosity for the X3CR1 M280 allele appears to be associated with increased risk of AMD.[44] Collectively, these and many other studies implicate disruption in immune function in the retina in the pathogenesis of AMD.

In this study, it was not possible to observe the hemosiderin-containing macrophages in the intact eye, due to their optical properties, and so longitudinal analysis in individual animals was precluded. However, hemosiderin can be formed in macrophages only after a hemorrhage has occurred. Because the number of observed hemorrhages and the numbers of patches of hemosiderin-containing macrophages remain relatively constant (as indicated in Table 1) and equivalent in incidence throughout life, it is proposed that the macrophages observed at later ages are the same population as those observed immediately after the early hemorrhages.

The radial relationship between macrophage infiltration in the inner retina and subsequent events in the outer retina may be a consequence of the functional activation of the Müller cells (as evinced in this study by changes in GFAP and GLAST expression). The structural integrity of Müller cells also appears to be a key determinant in photoreceptor survival, since GFAP- or vimentin-deficient mice exhibit reduced photoreceptor loss after retinal detachment.[45] Accordingly, perturbation of Müller cell function over an extended period may account for the radially targeted nature of retinal degeneration after an earlier hemorrhage. Glial responses have also been noted when autologous blood is injected into the vitreous humor, evoking the formation of glia-derived structures resembling epiretinal membranes that may be present in AMD.[46,47]

The mixture of features described in this study, such as BRB breakdown, focal photoreceptor loss, reactive glial changes, and neovascularization are hallmark features of AMD. One feature of AMD that is not entirely replicated in this model is the formation of drusen that are located between Bruch's membrane and the RPE cells. The autofluorescent drusenlike accumulations that are detected in wholemounts of the retina appeared to be comparable to the drusenlike fluorescent inclusions noted in other rodent models when similarly viewed.[43] However, analysis of transverse sections through the drusenlike bodies observed in this study suggest that at least some of the components of these fluorescent bodies represent autofluorescent debris formed during degeneration of the outer retina and macrophages. Whether unequivocal drusenlike structures may be formed over longer periods remains to be determined.

It is concluded that the animals identified in this study represent a novel rodent model of neonatal hemorrhage. Because hemorrhaging is a common event, both in the retinas of neonates and also in aged humans, the findings reported are

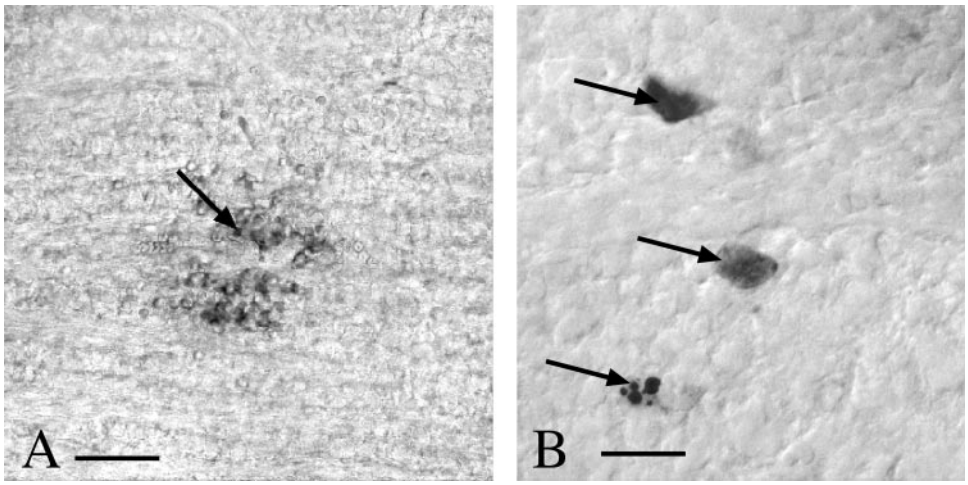

FIGURE 8. (**A**) Human fetal retina (18.5 weeks' gestation), showing a small retinal hemorrhage (*arrow*) on the vitread surface of the central retina. (**B**) Intense staining for hemosiderin was present in macrophages (*arrows*). Scale bars, 50 μm.

likely to have direct physiological relevance. The novel linkage identified between events in the neonate and latent events in the aged animal may have direct relevance to our understanding of the pathogenesis of human disease and, accordingly, requires further examination, both in this animal model, and in human tissues. Our current hypothesis, based on the data presented is that events early in life (or indeed later in life) may create a latent long-term propensity for neurodegenerative events in the retina and possibly other areas of the central nervous system.

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